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Physiological status of cow breeds Blonde d'Aquitaine, Chianina, and Marchigiana, which undergo a transition from summer to winter feeding

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Abstract

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The biochemical parameters of the blood of 23 cows of the Blonde d'Aquitaine (n-13), Chianina (n-5), and Marchigiana (n-5) cattle breeds at the same age, and in the same physiological and bodily condition were examined at the end of the grazing period. The animals were reared on the same farm, on natural pastures, and were not given additional concentrated feed. The breed had a significant influence on the levels of glucose ($P < 0,01$), Alkaline Phosphatase ($p<0,01$), Carbon Dioxide ($P<0,05$), and Urea Nitrogen ($P < 0,001$)/ Creatinine ($P < 0,001$) ratio. The Chianina cows exhibited the best physiological parameters and the highest adaptability to the conditions. The Marchigiana cows exhibited alimentary discomfort associated with lower glucose levels (2,992±0.225), higher creatinine levels (146.4±14.67), and lower BUN (2,026±0.090) and BUN/Crea ratio levels (14.75±1.688). The worst physiological markers, demonstrating poor digestion of the pasture grass at the end of the grazing period and starvation, were exhibited by the Blonde d'Aquitaine breed- glucose (2,255±0,143), BUN (1,152±0,055), BUN/Crea (8,764±0,821). Higher total blood protein (85,15- 88,50 g/L) against high globulin (57,03- 57,42) and tCO_2 (27,23-29,81), and negligibly lowered Na levels were reported for the cows of the three breeds. The remaining parameters tested, including ALB, TB, Crea, ALT, AMY, ALP, Ca, P, Mg, K, and Cl-, were within physiological norms.

Keywords: Beef cattle; feeding; grazing; biochemical analysis

Introduction

The climatic changes associated with global warming are expected to impact beef cattle breeding negatively. Rising temperatures are associated with an increased risk of heat stress and alimentary distress, change in the disease etiologies, draught in pasture areas (Scholtz et al., 2013; Brown-Brandl, 2018) and, as a whole, a negative influence on the health, productivity and wellbeing of the cattle (Brown-Brandl, 2018; Lees et al., 2019). To eliminate the negative consequences, it is necessary to apply new and more sustainable strategies when selecting animals that are better adapted to the respective production system (Scholtz et al., 2013; Mee and Boyle, 2020). Apart from its better adaptive ability, the cost-efficient production of meat from specialized beef cattle requires the breed to offer high productivity, regular reproduction, and economic efficiency in its rearing (Greenwood, 2021). Pasture farming is primarily used in beef cattle farming because it is not only considered the most beneficial and biocompatible for the cattle (Spigarelli et al., 2020; Smid et al., 2020), but it is also regarded as a technology that positively influences meat quality (Del Campo et al., 2020). The pastures promote the animals' wellbeing through the botanical composition of the grass and the climatic conditions, which are both directly connected to the nutrient balance, oxidation stress, and behaviour (Nakajima and Yayota, 2019). However, the pasture farming is also associated with a certain degree of discomfort experienced by the an-

imals, malnutrition (Mee and Boyle, 2020), concerns about possible chronic hunger and thirst, thermal stress (Temple and Manteca, 2020), higher internal parasitism risk, slower reproductive capacity recovery after birth (Mee and Boyle, 2020) etc. According to Aubé et al. (2022), the cows' welfare may vary throughout the grazing season due to changes in meteorological conditions, the quality of the pasture grass, and other factors. The biochemical parameters of the blood are considered indicative of the health and physiological condition of the animals (Zaitsev et al., 2020) and are therefore used to assess both them and the stress reaction (Berian et al., 2019; Kim et al., 2021; Wang et al., 2023) and as criteria for the humane treatment of animals (Radkowska and Herbut, 2014; Loi et al., 2021) etc. According to Bobbo et al. (2017), if interpreted correctly, the serum protein levels may serve as a potential indicator for the animal's well-being. Tarantola et al. (2020) report significant differences in protein metabolism, as indicated by changes in albumin, total protein, and creatine kinase plasma concentrations, in animals reared in different production systems. They recommend the parallel use of animal condition data and blood analyses for assessing their well-being. The present study aims to compare the biochemical blood profile of introduced beef cattle breeds, reared on natural pastures, at the end of the grazing period.

Materials and Methods

The study was performed in 2021, and is part of the National Science Program INTELLIGENT ANIMAL HUSBANDRY, which focuses on the use of IoT systems for the monitoring of the behavior, reaction to stress factors, and evaluation of the physiological and health status in cattle about designing environmentally friendly and organic cattle farming technologies and optimal exploitation of the natural resources. According to Herlin et al. (2021), pasture farming using automatic equipment allows for constant monitoring, providing more information than direct monitoring. For the monitoring system to be fully developed, it is first necessary to obtain a comprehensive volume of information regarding the biological characteristics of the animals and their dynamics under various environmental factors. In this respect, we studied the biochemical blood parameters of 23 cows from specialized beef cattle breeds —Blonde d'Aquitaine (n = 13), Chianina (n = 5), and Marchigiana (n = 5) — at the end of the grazing period. The animals were of the same age (5-6 years) and had identical physiological status (non-pregnant, with weaned calves) and bodily condition, graded at five on a 9-point scale. The cows were progeny of introduced parents from France and Italy. The animals were reared under the same conditions in a farm located near the town of Lom in the Montana region. The cows were reared on natural pastures along the River Danube from May to November. During the grazing period, the cows were not given additional concentrated feed. The pasture terrain was plain; however, due to the early droughts, the grass started drying in August. The blood for testing was drawn from the caudal vein using lithium heparin vacuum test tubes. The hematological parameters were examined within 24 hours after obtaining the samples using an automatic biochemical analyser, Seamaty SMT-120V. The reagent disks used during the analysis covered major biochemical parameters of the blood– Glucose (GLU), Total Protein (TP), Albumin (ALB), Total Bilirubin (TB), Creatinine (Crea), Urea Nitrogen (BUN), Total Carbon Dioxide/Bicarbonate (tCO_2), Calcium (Ca), Phosphorus (P), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), Amylase (AMY). The Globulin (GLOB=TP-ALB) and Albumin/Globulin (AG) ratio were automatically calculated. The values for 'cows' as set in the biochemical analyser software were accepted as reference values. The data were statistically analyzed using specialized software (SPSS 21, IBM). The following statistical model was used: $Yij = \mu + Bi + eij$, where B is the breed (n = 3) and eij are the residuals. The differences between the groups were compared using Tukey's test.

Results

Overall, the animals of the three breeds displayed similar values of the major biochemical parameters. A significant influence of the breed was ascertained only for 5 of the 19 parameters tested: glucose levels, alkaline phosphatase levels, urea nitrogen, creatinine ratio, and carbon dioxide quantity (Table 1).

Table 1. Influence of the breed on biochemical blood parameters of cows at the end of the pasture period

Parameters	F-criteria and confidence	Parameters	F-criteria and confidence
GLU	10.02**	BUN	12.95***
TP	0.566	BUN/Crea	18.06***
ALB	0.168	tCO_2	3.868*
GLOB	0.007	Ca	0.623
AG	0.033	Phos	0.999
TB	0.715	Mg	0.310
ALT	0.844	K	0.488
ALP	2.639*	Na	0.323
AMY	0.572	Cl	0.702
Crea	2.314		

***p < 0.001; ** p < 0.01; * p < 0.05

The lowest glucose levels were reported for the Blonde d'Aquitaine cows. They were 38,4% lower than those of the Chianina ($P < 0.001$) and 24,6 % higher than those of the Marchigiana cows (Table 2). Perhaps that was the reason why the difference in glucose levels between the Chianina and Marchigiana breeds (18.3%) was unreliable and relatively large. The glucose levels of the latter two breeds were within physiological norms, while those of the Blonde d'Aquitaine cows were 11.9% lower than the lower limit of the reference range.

The urea values for Blonde d'Aquitaine were considerably lower, as indicated by the urea nitrogen (BUN) levels ($P < 0.01$) compared to those for the Chianina and Marchigiana breeds, which were twice as high. This also led to reliably lower BUN/Crea ratio values reliably, respectively, 55.7% ($P < 0.001$) compared with the Chianina and 40.6% ($P < 0.01$) compared with the Marchigiana, even though the creatinine level was within the reference norm and was the lowest in the Chianina. All three breeds exhibited BUN values that were lower than the reference ones. Along with the low glucose levels, the Blonde d'Aquitaine also had higher total bilirubin levels, which were 28.6% higher compared to those of the Chianina and 15.0% higher than those of the Marchigiana. However, although it was close to the lower reference value, the parameter was within the reference range for all three breeds. The carbon dioxide values were lower for the Blonde d'Aquitaine, and the 8.65% difference from the Chianina was reliable ($P < 0.01$). The parameter values of the Blonde d'Aquitaine, however, were closer to the optimal ones when compared with the values of the Chianina and Marchigiana because the values of all three breeds were above the upper limit of the reference range. The total protein levels of the three breeds were above the upper reference limit, ranging from 6.43% for the Blonde d'Aquitaine to 10.6% for the Marchigiana. The increase was at the expense of only the high globulin levels (50% above the upper reference limit), as the albumin level was within the reference range. The ALT concentration in the Blonde d'Aquitaine blood was 29,7% higher than that of the Chianina and and 23,5% higher than the Marchigiana (Table 3). However, the differences between the breeds were unreliable and within the reference values. The AMY concentration was the lowest in the blood of the Blonde d'Aquitaine cows. Its values were similar to those of the cows of the other two breeds, and, regarding all three breeds studied, they were close to the average values of the upper reference limit. Unlike the case of ALT and AMY, there were considerable differences in alkaline phosphatase levels. The ALP levels of the Chianina cows were 2,16 times

Table 2. Biochemical Profile of the Blood of Blonde d'Aquitaine, Chianina and Marchigiana Cows

Parameter	Breed	N	LS	Sx	SDev	Ref	
						min	Max
GLU, mmol/L	BL	13	2.255[a]	0.143	0.495	2.56	5.18
	CH	5	3.662[a]	0.535	0.927		
	MA	5	2.992	0.225	0.451		
ALB, g/L	BL	13	28.80	1.311	4.543	25.0	36.0
	CH	5	29.20	1.805	3.127		
	MA	5	30.04	1.447	2.894		
TP, g/L	BL	13	85.15	1.214	4.206	58.0	80.0
	CH	5	86.62	5.616	9.728		
	MA	5	88.50	3.519	7.038		
GLOB, g/L	BL	13	57.03	1.545	5.355	27.0	38.0
	CH	5	57.37	6.521	11.29		
	MA	5	57.42	3.488	6.976		
A/G	BL	13	0.513	0.034	0.121		
	CH	5	0.531	0.084	0.145		
	MA	5	0.522	0.029	0.058		
TB, umol/L	BL	13	4.507	0.540	1.873	0.0	12.0
	CH	5	3.500	0.697	1.208		
	MA	5	3.920	0.258	0.516		
Crea, umol/L	BL	13	139.3	9.194	31.84	0.0	172.0
	CH	5	106.4	12.81	22.19		
	MA	5	146.4	14.67	29.34		
BUN, mmol/L	BL	13	1.152[bc]	0.055	0.191	2.5	6.1
	CH	5	2.155[b]	0.563	0.972		
	MA	5	2.026[c]	0.090	0.181		
BUN/Crea	BL	13	8.764[ac]	0.821	2.846	19	202
	CH	5	19.77[a]	2.899	5.022		
	MA	5	14.75[c]	1.688	3.376		
tCO_2	BL	13	27.23[b]	0.519	1.801	21.0	27.0
	CH	5	29.81[b]	1.007	1.745		
	MA	5	28.22	0.427	0.855		

The differences between the groups with the same index for the respective parameter are reliable a- $P < 0.001$; bc- $P < 0.01$%

Table 3. Levels of some enzymes in the blood of cows of the Blonde d'Aquitaine Chianina and Marchigiana breeds

Parameter	Breed	N	LS	Sx	SDev	Ref	
						min	Max
ALT, U/L	BL	13	26.92	2.966	10.27	5.0	35.0
	CH	5	20.75	3.175	5.501		
	MA	5	21.80	5.813	11.62		
AMY, U/L	BL	13	64.30	5.729	19.84	41	98
	CH	5	73.25	8.684	15.04		
	MA	5	71.60	4.919	9.838		
ALP, U/L	BL	13	43.84	5.800	20.09	0.0	149.0
	CH	5	95.00	48.68	84.33		
	MA	5	54.41	12.90	25.80		

higher than those of the Blonde d'Aquitaine and 74,6% higher than those of the Marchigiana. The unreliability of the differences was probably linked to the significant individual variation in the Chianina cows' ALP values.

At the end of the grazing period, the pasture provided optimal quantities of macronutrients, and their assimilation did not differ reliably (Table 4). A negligible deficit was reported for Na and Cl, but these minerals could be easily supplied by administering NaCl.

Table 4. Levels of some macronutrients in the blood of cows of the Blonde d'Aquitaine Chianina and Marchigiana breeds

Parameter	Breed	N	LS	Sx	SDev	Ref	
						min	Max
Ca, mmol/L	BL	13	2.185	0.037	0.130	1.95	2.62
	CH	5	2.187	0.050	0.086		
	MA	5	2.254	0.056	0.112		
Phos, mmol/L	BL	13	1.756	0.077	0.268	1.38	2.55
	CH	5	1.992	0.241	0.417		
	MA	5	1.888	0.165	0.331		
Mg, mmol/L	BL	13	0.780	0.067	0.232	0.53	1.00
	CH	5	0.710	0.093	0.162		
	MA	5	0.816	0.061	0.123		
K, mmol/L	BL	13	5.677	0.149	0.516	3.90	5.80
	CH	5	5.410	0.299	0.519		
	MA	5	5.838	0.500	1.000		
Na, mmol/L	BL	13	124.6	1.173	4.066	132.0	152.0
	CH	5	125.8	2.466	4.271		
	MA	5	126.2	2.057	4.116		
Cl, mmol/L	BL	13	96.26	2.039	7.063	97.0	111.0
	CH	5	100.3	4.131	7.156		
	MA	5	96.08	0.616	1.233		

The blood count we observed gave us grounds to conclude that, irrespective of their identical bodily condition, the cows of the Blonde d'Aquitaine, Chianina, and Marchigiana breeds considerably differed in their physiological status at the end of the grazing period. The best physiological parameters and the highest adaptability to the environment were exhibited by the cows of the Chianina cattle breed, followed by those of the Marchigiana. The latter exhibited a certain alimentary discomfort associated with glucose levels that were close to the lower limit of the reference range, as well as higher creatinine and lower BUN and BUN/Crea ratio values. The Blonde d'Aquitaine cows had the most negative physiological blood markers. The values of these breed parameters examined were indicative of imperfect pasture grass assimilation at the end of the grazing period and starvation. Upon analyzing the results, we excluded fasciolosis infestation which manifests with hypoglycemia (El-Aziem Hashem and Mohamed, 2017), high globulin levels (Frejuk and Stybel, 2021), increased creatinine levels (Nasreldin and Zaki, 2020), lower A/G ratio (Frejuk and Stybel, 2021) as increased ALT (Nasreldin and Zaki, 2020; Brahmbhatt et al., 2021) and ALP levels (Lotfollahzadeh, et al., 2008), accompanied with hypoproteinemia, hypoalbuminea (El-Aziem Hashem and Mohamed, 2017; Brahmbhatt et al., 2021; Frejuk and Stybel, (2021)) and considerable increase in the total bilirubin (Mircheva, 2006; Brahmbhatt et al., 2021), which are all typical for parasitosis, were not reported. Kalugniy et al. (2021) confirm the significance of the bilirubin in the diagnostics and note that in cattle, it may reach upper limit values up to and over 16,89 ± 2,51 mmol/l. The authors have reported TB–5,17 ± 2.26 mmol/L, ALT–12.43 ± 2.72 IU/L, and AST–93.52 ± 3.52 IU/L in healthy heifers of the Holstein cattle breed. The low blood glucose levels are a sign of malnutrition. During a trial with calves, Ortolani et al. (2020) ascertained a reliable negative correlation (-0.52, $p < 0.0001$) between the period of fasting and the blood glucose concentration. Glucose levels below 40 mg/dL (2,23 mmol/L) are considered hypoglycemic for dairy cows (Mair et al., 2016), which generally have lower values. We reported glucose levels similar to those mentioned concerning the Blonde d'Aquitaine. Megahed (2028) even defines values below 55 mg/dL (3,0 mmol/L) as hypoglycemic for cattle. Mullinikis et al. (2019) state that beef cattle adapt to environmental changes by adjusting their metabolic use of energy. According to the authors, this mechanism involves adaptive processes that lead to changes in nutrient distribution and energy use efficiency. Glucose is absorbed directly by the digestive tract or obtained during glycogenesis, which is the primary method for ruminants (Reynolds, 2005; Aschenbach, 2010). Upon insufficient glucose in the feed, it is synthesized from the metabolites of body fats and proteins through glycogenesis (Rui, 2014; Gross et al., 2013; Xu et al., 2020; Ortolani et al., 2020). Some of the reasons for the lowering of urea levels in the blood of people, which we also observed in the Blonde d'Aquitaine cows, include a low-protein diet, malnutrition, or starvation (Walker et al., 1990). An increased burning of amino acids by starving cattle, which require energy, was observed in the study by Ortolani et al. (2020) mentioned above. Walker et al. (1990) note that urea and creatinine are nitrogen metabolism end products, with urea being the major metabolite derived from food protein and tissue protein metabolism, and creatinine a product of muscle creatinine catabolism. The authors note that the low BUN/Cr ratio (which in the Blonde d'Aquitaine was twice the lower reference limit) may indicate inadequate protein intake. Salazar (2014) claims that the urea concentration (which in the Blonde d'Aquitaine was also two times lower than the lower reference limit) depends on the protein intake, the body's ability to catabolize protein, and the adequate release of urea from

the renal system. The authors note that, when compared with BUN, creatinine is less affected by dietary changes.

Based on the application of a 'RIBECA' system protocol and analysis of the biochemical parameters of the blood, Tarantola et al., (2020) have ascertained that Blonde d'Aquitaine bulls have better adaptability than the Piedmontese cattle and beef breeds crosses upon intensive free-range and group farming and report the following blood parameters values- glucose 106,85 ± 5,91 mg/dL (5,93 mmol/L), total protein 6,62 ± 0,09 g/dL, albumin – 3,75 ± 0,005 g/dL (37,5 g/L), creatinine – 2,01 ± 0,09 mg/dL (112 umol/L). Similarly, in an earlier study, Nikolov et al. (2022) reported low blood glucose levels at the end of the grazing period, 2,062±0.082-2,399±0.130 mmol/L, for cows of the autochthonous Rhodope Shorthorn cattle reared on two farms with semi-mountainous pastures. Significantly lowered BUN-1,601±0,110 mmol/L and BUN/Crea ratio- 15,22±0,992 values were observed in one of the farms. Obviously, at the end of the grazing period, the natural pastures in Bulgaria, both in the plains and in the semi-mountainous regions, cannot provide for the essential nutrient needs of the cattle.

Conclusion

After a comparative analysis of the blood biochemical parameters, it was ascertained that, irrespective of their identical bodily condition, the cows of three introduced beef breeds – Blonde d'Aquitaine, Chianina, and Marchigiana —reared in the same farm, differed significantly in their physiological status at the end of the grazing period. A reliable influence of the breed was observed in the levels of glucose ($P < 0.01$), alkaline phosphatase ($P < 0.01$), urea nitrogen ($P < 0.001$), and its creatinine ratio ($P < 0.001$), as well as the quantity of carbon dioxide ($P < 0.05$). The best physiological parameters and the highest adaptability to environmental conditions were displayed by the cows of the Chianina breed, followed by those of the Marchigiana breed. The latter showed a certain alimentary discomfort connected to the blood glucose levels, which were close to the lower reference limit (2,992±0,225), the higher creatinine (146,4±14,67), the lower BUN (2,026±0,090), and the BUN/Crea ratio (14,75±1,688) levels. At the end of the grazing period, the worst physiological blood markers, indicative of poor assimilation of the pasture grass and starvation, were reported for the Blonde d'Aquitaine cattle breed: blood glucose (2,255±0.143), BUN (1,152±0.055), and BUN/Crea (8,764±0.821). The tree breeds all exhibited increased total protein levels (85.15-88.50 g/L), high globulin (57.03-57.42), tCO_2 (27.23-29.81), and insignificantly lowered Na levels. The rest of the parameters tested, including ALB, TB, Crea, ALT, AMY, ALP, Ca, P, Mg, K, and Cl, were all within the reference range values. Our present and previous studies show that the natural pastures in Bulgaria, both in the plains and in the semi-mountainous regions, cannot meet the essential nutrient needs of cattle at the end of the grazing period.

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